Trail Pheromones: Responses of the Texas Leafcutting Ant, *Atta texana*[1] to Selected Halo- and Cyanopyrrole-2-Aldehydes, Ketones, and Esters[2]

P. E. SONNET[3] AND J. C. MOSER[4]
USDA

ABSTRACT

Several halo- and cyanopyrroles related to the trail pheromone of *Atta texana* (Buckley), were prepared and tested by a faster and more sensitive bioassay than was previously available. Responsiveness of the ants in descending order to these compounds, based on the substituent in the number two position, is: esters, methyl ketones, aldehydes. Slight activity was observed when the nitrogen atom of the pyrrole ring was alkylated.

The recent description (Tumlinson et al. 1972) of a trail pheromone of the Texas (town) leafcutting ant, *Atta texana* (Buckley), represents the 1st elucidation of an ant trail substance. Synthetic work directed toward developing active analogues and toward understanding the (chemical) structural basis for activity would be valuable for arriving at a selective control method devoid of toxicants. Although the town ant, a nuisance primarily because of its wintertime destruction of pine seedlings, is the only established leafcutting ant species in the United States, there are 180–200 species of leafcutting ants in this hemisphere, many of which rank as major economic pests in Central and South America (Weber 1972). Knowledge and methodology gained from *A. texana* may be useful in future attempts to control other species of the tribe Attini.

Sonnet recently developed an excellent synthetic route to 4-substituted pyrrole-2-aldehydes and esters (Sonnet 1971, 1972a). When this method was applied to obtain screening candidates (Sonnet and Moser 1972) we discovered that the 4-chloro and 4-bromopyrrole-2-carboxaldehydes and methyl carboxylates were quite active. On the other hand, positional isomers of the natural pheromone, methyl 4-methylpyrrole-2-carboxylate (*1*) were essentially inactive, as was the N-methyl derivative of *1* (compounds are identified in Table I). We surmised from this fact that the carbonyl substituent must be adjacent to an unsubstituted nitrogen for maximum activity and that the identity of the other ring substituent was of lesser consequence than its position on the ring. We undertook preparation and examination of a series of halogenated esters, aldehydes, and ketones, and we describe here the responses of the town ant as measured by a new and more efficacious bioassay.

Materials and Methods

The syntheses of the pheromone, *1* (Sonnet 1972b), and of the halogenated esters and aldehydes, *2*, *3*, *6*, and *7*, as well as of *13*, and *22* (Sonnet 1972a, Sonnet and Moser 1972) have been described. The preparations of the iodo and cyano derivatives, *4*, *5*, *8*, *9*, *14*, *15*, and *18* also are described in detail elsewhere (Sonnet 1973). The halogenated ketones (*10–12*) and the pyrrolizinones (*19–21*) will likewise be detailed in an appropriate chemical publication. Compounds *16* and *17* were prepared by *N*-methylation (sodium hydride followed by methyl iodide) of *6* and *7* respectively.

All structures were confirmed by infrared and nuclear magnetic resonance spectroscopy, chemical analyses, and gas chromatography whenever the possibility of positional isomerism existed. For example, *16* yielded the following physical data: melting point 46.5–48°C (from petroleum ether); a carbonyl band at 5.95 μ but no NH band at 5.95 μ and no NH band in its infrared spectrum, and the nuclear magnetic resonance spectrum which was observed in carbon tetrachloride (δ values are given in parts per million from tetramethylsilane) showed bands at 3.87s(N-CH_3), 6.72 m (aryl H's) and 9.43s(CHO). The aldehyde absorption was broadened by the expected long-range coupling to one of the aryl protons (Gronowitz et al. 1961). In addition a chemical analysis corresponding to a molecular formula of C_6H_6ClNO was obtained.

Bioassays were performed by using the lost-ant technique, a method that is at least 10 times more sensitive than the "minor worker bioassay" previously used by us. This new bioassay was first developed by S. W. Robinson, Leaf-cutting Ant Research Unit, University College of North Wales, Bangor, U.K. (personal communication). The present method is a modification of Robinson's original idea.

In the lost-ant bioassay, a 10-μl chloroform solution of the candidate chemical is used to describe a 50-cm circular circumference on cardboard. This cardboard is used in the same way as in the previous technique (Moser and Blum 1963; Sonnet and Moser 1972). However, the minor workers are not taken from a colony and placed in the center of the circle. The cardboard is simply placed on the floor of a large ant cabinet (plexiglass box) where workers are already circulating (Fig. 1). The technique works best when a fungus garden with ants is placed in the box immediately after being brought in from the field. This releases immediate searching behavior,

[1] Hymenoptera: Formicidae.
[2] Received for publication 5 Apr. 1973.
[3] Organic Chemicals Synthesis Laboratory, Agricultural Environmental Quality Institute; Beltsville, Md. 20705.
[4] Southern Forest Experiment Station, Pineville, Louisiana, 71360.

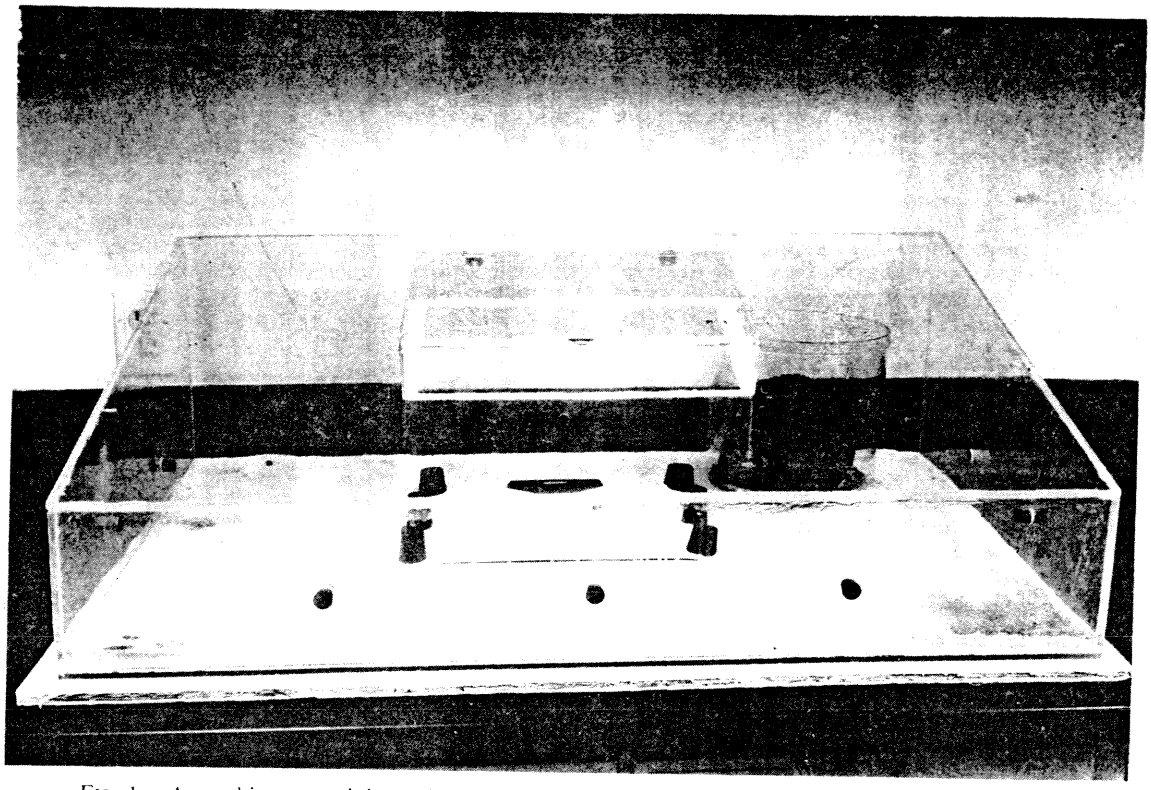
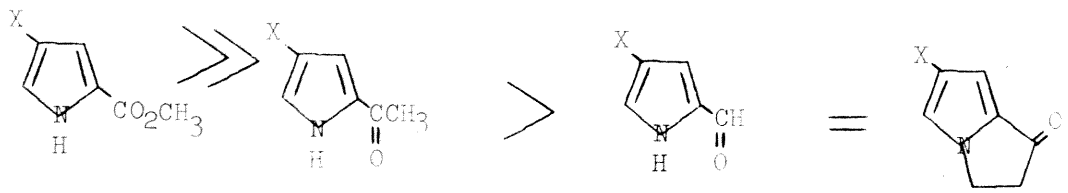


FIG. 1.—Ant cabinet containing a fresh fungus garden (on the right) and a cardboard test sheet.

providing a maximum number of ants available for finding the artificial trail on the cardboard. The bioassay is most efficient within 3 h after the garden is introduced. After that time the ants become progressively more lethargic.

Forty or more bioassays are possible during a 3-h period—far more than with the old technique. Another advantage is that all sizes of workers respond to the bioassay because the alarm factor is eliminated. By the old method only minor workers could be used, because larger sizes were too excitable when introduced into the center of the circle. Moreover, the larger ants appear to be more efficient searchers than the minor workers.

As many as 100 ants may be milling on the card at a given time, making precise counts impossible. Therefore, scoring is denoted by strong, medium, and weak. A strong response is obtained when 50% or more of the workers follow the trail for at least 10 cm during a 5-min period. A medium response is when about 10% follow, and a weak response if only 2 or 3 ants definitely follow. (Because of the damp cement base, weights are needed to keep the cardboard from curling.)



X = halogen

FIG. 2.—Relative activities of the halogenated pyrroles on *A. texana*.

Results and Discussion

Table 1 shows the compounds examined and the responses of the town ant to the 3 concn employed. Most values are averages of several tests. The lost-ant technique is sufficiently sensitive that even the lowest concentrations of *1–3* gave strong responses. Interestingly, the aldehydes *6–8* are not more active by this new assay method, and appear, therefore, to be considerably less active than the esters. Replacement of a halogen by CN causes a loss of activity in all cases examined (compare *5* with *3*, and *9* with *7*). Fig. 2 compares activities of the various types of carbonyl compounds.

More interesting are the results obtained with pyrroles bearing substituents on nitrogen. The N-methyl derivatives, Compound *13*, of the pheromone (purified by gas-liquid chromatography) repeatedly elicited strong responses at the highest concentration tested. In general, N-methyl derivatives did produce the trail-following behavior at this concentration. The activity of the aldehydes *16* and *17* is about the same as that of the unmethylated analogues *6* and *7*. Moreover, compounds in which the nitrogen substituent was secured to the acetyl group to form the pyrrolizinones *19–21* resulted in a similar level of activity. The method of synthesis for such compounds makes the presence of even traces of free NH highly unlikely.

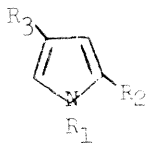
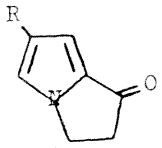
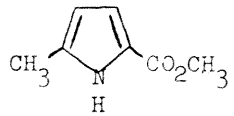
In summary, greatest activity was obtained with compounds having a free NH, an adjacent ester function, and a substituent at ring position 4. While steric requirements are stringent at the carbonyl site (Sonnet and Moser 1972), they are surprisingly liberal at both the nitrogen and methyl-bearing carbon atoms. Even though some activity persists with compounds such as *13* and *20*, the inactivity of the positional isomer, *22*, attests to the requirement of a 1, 2, 4-relationship between nitrogen, carbonyl, and the other substituent.

The carbonyl group of the pyrrolizinones is frozen in the *transoid* configuration and this fact leads naturally to speculation as to the response of *A. texana* to the *cisoid* analogue *23* (See Fig. 3). We hope to examine such compounds in the future.

transoid cisoid

FIG. 3. — Fixed carbonyl configurations.

Table 1.—Trail-following responses of the town ant to pyrrole derivatives.

Compound no.	Structure			Concentration (ng/μl) vs.		Average rating[a]
				40	0.4	0.004
	R_1	R_2	R_3			
1	H	CO_2CH_3	CH_3	—	—	S
2	H	CO_2CH_3	Cl	—	—	S
3	H	CO_2CH_3	Br	—	—	S
4	H	CO_2CH_3	I	—	—	M
5	H	CO_2CH_3	CN	M	W	O
6	H	CHO	Cl	M	O	O
7	H	CHO	Br	W	O	O
8	H	CHO	I	W	O	O
9	H	CHO	CN	O	O	O
10	H	$COCH_3$	Cl	S	W	O
11	H	$COCH_3$	Br	M	O	O
12	H	$COCH_3$	I	M	O	O
13	CH_3	CO_2CH_3	CH_3	S	O	O
14	CH_3	CO_2CH_3	CN	M	O	O
15	CH_3	CHO	CN	O	O	O
16	CH_3	CHO	Cl	W	O	O
17	CH_3	CHO	I	O	O	O
18	CH_3	CHO	I	O	O	O
						
19		Cl		W	O	O
20		Br		M	O	O
21		I		W	O	O
22						

[a] S, strong; M, medium; W, weak; O, no response.

REFERENCES CITED

Gronowitz, S., A. B. Hornfeldt, G. Bestblom, and R. A. Hoffman. 1961. On the coupling constants of pyrroles. Ark. Kemi. 18: 133–41.

Moser, J. C., and M. S. Blum. 1963. The trail marking substance of the Texas leaf-cutting ant. Source and potency. Science (Wash., D. C.) 140: 1228.

Sonnet, P. E. 1971. Synthesis of β-substituted pyrroles *via* 1-(pyrrol-2-ylmethylene) pyrrolidinium salts. J. Org. Chem. 36: 1005–7.

1972. Preparation and properties of ternary iminium salts of pyrrole aldehydes and ketones. Synthesis of 4-substituted pyrrole-2-carboxaldehydes. Ibid. 37: 925–9.

1972b. Synthesis of the trail marker of the Texas leaf-cutting ant, *Atta texana* (Buckley). J. Med. Chem. 15: 97.

1973. 4-Iodo and 4-cyanopyrrole-2-carboxaldehydes. J. Heterocycl. Chem. 10: 113–4.

Sonnet, P. E., and J. C. Moser. 1972. Synthetic analogs of the trail pheromone of the leaf-cutting ant, *Atta texana* (Buckley). J. Agric. Food Chem. 20(6): 1191–4.

Tumlinson, J. H., J. C. Moser, R. M. Silverstein, R. G. Brownlee, and J. M. Ruth. 1972. Volatile trail pheromone of the leaf-cutting ant, *Atta texana*. J. Insect Physiol. 18: 809–14.

Weber, N. A. 1972. Gardening Ants. The Attines. Mem. Am. Philos. Soc., Phila. 92: XVII; 1–146.

Reprinted from the
ENVIRONMENTAL ENTOMOLOGY